

Proteome Analysis of Desulfovibrio vulgaris upon Salt Stress Using 3D LC-MS/MS

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Abstract

Salt is one of the stress factors for anaerobes like Desulfovibrio vulgaris in the environment. NaCl is found in high concentrations at many DOE contaminated sites. The exact stress response pathways of D. vulgaris induced by NaCl are not known. To better understand the cellular responses to salt stress, we studied the proteome composition and expression levels of D. vulgaris upon exposure to NaCl at 250mM for approximately two hours. Whole cell proteomes were extracted from cells before and after treated with NaCl or H2O. Protein compositions were assessed using the 3D LC-MS/MS technology developed at Diversa Corporation. Proteins corresponding to over 50% of the predicted 3,500 ORFs were identified among the three samples. Relative protein abundance in each of the samples were represented by the spectrum counts for each identified protein. Lists of candidates that are potentially involved in salt stress response were obtained. The significant differences for proteins to be either up- or down-regulated were evaluated by the Local Pooled Error (LPE) test. The proteomic results were also compared to the ones from the same set of samples analyzed by alternative profiling techniques. The 3D proteomic technology provides tools for detailed mapping as well as global view of gene expression at protein levels during the stress response in D. vulgaris.

Goals and Solutions

Goals

- Global view of proteome and changes in protein expression upon salt stress
- Rapid one-fraction protocol for protein
- extraction
 3D LC MS/MS for comprehensive peptide and protein identification
- Statistical tests based on the duplicated analysis to determine significant changes

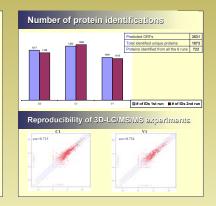
Control Salt 250 mM T = 0 h C0 V0 T = 2 h C1 V1 > Samples were collected at C0. C1 and V1 states: > The same biomass was used to conduct transcriptome and proteomic profiling experiments:

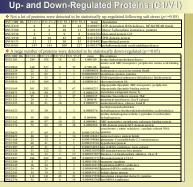
Each sample was run in duplicates to understand the reproducibility of the

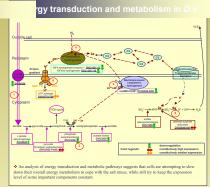
**O.5 to 1 million MS/MS spectra per injection * Minimal sample handling/ SDS tolerance **Outst Lend Intermited Content with Liquid (1.8 kV) **Reverse Phase Resin **Reverse Phase

· Patent Pending

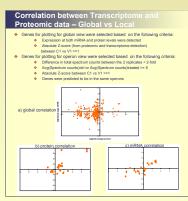
Culture 10-20 ml cells 1% RapiGest" Centrifugation Whole cell lysate 350 up proteins Protease digestion Hydropholic peptides 3D LC-MS/MS a 3D LC-MS/MS b Data













	O ₃ Stress				Salt Stress				Nitrato Stress				
Up-trends													
DVU IIDs	Cl_1	C1_2	VIJI	V1_2	CI-I	C1-2	V1-1	V1-2	CI-I	C1-2	VI-I	V1-2	Descriptions
DVU0774	.33	- 11	20	41	- 10	30	23	27	4		22	- 19	ATP synthese, F1 spellers subsent
DVU0775	721	697	1962	201	291	371	333	426	279	201	30.0	411	ATP synthese, F1 bris subsent
DVU0790	114	21	104	17	62	26	12	79	ax.	31	.19	40	ATP synthese FG, If subsets, putative
DVU0835	41	31	100	71		29	-	24	- 1	7		- 11	obraseal perior L19
DVU1257	33	- 10	384	201	227	213	248	321	201	213	210	301	ENS binding protein
DVU1314		4	21	20		4	12	10	0.00	0.81	1	1.0	obsumal period 1.31
DVU1334	444	339	389	412	113	40	138	146	33	81	127	134	brigger States
DVU1470	39		m	39	10	62	132	37	40	47	33	67	popiskyli probjel sin insen inconsense C
DVUI574	339	203	664	311		193	280	179	199	200	199.	200	obsumal posicia 1.21
	33	21	138	41	39	29	27	33	10.	19	71	41	hypothetical protein
DVU1976	2266	1343	3770	2121	1233	1434	1010	1079	11107	1221	1766	100	obspectors, 60 Mile
DVU1977	248	10	204	101	33	28	47	28	82	44	.80	78	abspecials, 18 Mb
DVU2430	-1	- 1	39	39	- 1	1	3	4	2	1	4	. 1	EXX bading posicie
Down-t	rends												
DVU1611	31	16	0	7	- 4		0.01	0.01	13.0	2	- 1	93.0	molybidipteris cedoreductase dunais protein
DVU3002		- 60	2		- 1	3	2	0.01	2	- 1	0.01	- 1	hypothetical protein
DVU0855		- 4	2	- 4	- 1	2	- 1	2	10	13	- 1	- 1	radical SAM donain potein
DVU1441	36	21	21	- 11	30	31	11	12	9				Seption
DVU1922	67	er	37	24	- 11	12	7		13	17	٠,		perplaenic (NPV) bydrogenase, large subunit, successed 1
DVU2325	267	272	207	145	12		-	7	70	130	34	81	mercuric transport protein periplasmic component
DVU1949	- 11	- 1		7	13		0.01	3	12	- 19	- 1		nd specific regulatory protein
DVU1444	10	- 1	- 1	0	3	- 1	- 4	-	- 1	- 1	0.01	- 3	basel body rod modification protein FigD
DVU1443	14	25		16	100	116	102	65	31	24	3		Segetar hock protein FigS
DVLI3272	203	203	- 61	72	71	9.7	72	34	222	108	142	191	TPR donain potein
DVU0881	667	219	199	165	112	119	31	- 45	171	133	117	197	translation elongation factor Cl. putative
DVU1030	669	258	345	147	- 68	132	- 14	112	96		79	- 10	universal stress protein family
DVU0084	70		-		- 5	7	-	0.01	- 2	0.01	- 2	-	translation initiation factor, at F-281 family, published

Proteins show up- or down-regulated trends

Conclusions

- About 50% of the *D. Vulgaris* proteome can be assessed for expression changes
- More than 10% proteins displayed changes upon salt Stress
- Many of these proteins are components in the ABS transporter sytems
- Concerted changes in the protein levels observed along the same energy metabolic pathway

Acknowledgements

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